

Attorney Docket No. 950376D1/HG

**IN THE UNITED STATES PATENT  
AND TRADEMARK OFFICE**

Applicants : Tohru TAKAHASHI et al.  
Serial No. : (Divisional Appln. of  
Ser. No. 09/167,151)  
Filed : Concomitantly Herewith  
For : EXPRESSION SYSTEM  
UTILIZING AUTOLYZING  
FUSION PROTEINS AND A  
NOVEL REDUCING POLYPEPTIDE  
Art Unit :  
Examiner :

**PRELIMINARY AMENDMENT FILED  
CONCOMITANT WITH DIVISIONAL APPLICATION**

Assistant Commissioner for Patents  
Washington, D.C. 20231

S I R :

Please amend the application as follows.

**IN THE SPECIFICATION:**

Pages 12 and 13: replace the paragraph bridging pages 12 and  
13 with the following paragraph:

--The present invention will be illustrated with respect to  
the accompanying drawings, in which:

Figure 1 is a restriction enzyme map of cDNA of the NIa region  
isolated from CYVV-cDNA;

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Dorothy DeFrancesco

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Figure 2 is a schematic diagram which shows the construction of plasmid pKNI5' containing a 5'-region of NIa;

Figure 3 is a schematic diagram which shows the construction of plasmid pKNI5IL containing a part of the IL-11 gene and a 5'-region of NIa;

Figure 4 is a schematic diagram which shows primers which were used to prepare the 5'IL DNA fragment, the CIN3 DNA fragment and in which the 3'-terminus of the NIa gene and the 5'-terminus of the IL-11 gene are fused;

Figure 5 is a schematic diagram which shows the fusion of the CIN3 DNA fragment and the 5'IL DNA fragment by PCR;

Figure 6 is a schematic diagram which shows the construction of plasmid pKSUN9;

Figure 7 is a construction enzyme map of pUCKM31-7;

Figure 8 is a comparative diagram of the nucleotide sequences of the 3' terminals in pUCKM31-7 and pcD-31;

Figure 9 is a construction diagram of pSR $\alpha$ 31-7;

Figure 10 is a schematic diagram showing the introduction of a histidine hexamer encoding sequence into pUCKM31-7;

Figure 11 is a construction diagram for pMAL31-7;

Figures 12A and 12B are graphs showing the results of the assay of dichlorophenol-indophenol reducing activity; and

Figure 13 is a graph showing the determination of oxidized glutathione reducing activity.--

**Pages 99 and 100:** replace the paragraph bridging pages 99 and 100 with the following paragraph:

--The next step was to verify that the several specific 60 kDa bands identified in Example 11 are the same as the polypeptide encoded by the insert of pSR $\alpha$ 31-7. It was also desired to determine the N-terminal amino acid sequence of this polypeptide. Accordingly, a clone was prepared wherein an extra six His residues were encoded for the C-terminal of the polypeptide before the stop codon. Histidine residues have a high affinity for Ni<sup>2+</sup> and the objective was to express a polypeptide having a histidine hexamer (6 x His), which could be purified using an affinity resin column charged with Ni<sup>2+</sup>.--

**Pages 105 and 106:** replace the paragraph bridging pages 105 and 106 with the following paragraph:

--90.4  $\mu$ g, as determined using the Protein Assay Kit (Bio-Rad), of each of the chromatography samples obtained in ii) above were separately mixed with 1 ml of 50  $\mu$ M DCIP (Sigma). 15  $\mu$ l of 1 mM NADPH (Boehringer-Mannheim) were then added to each of the samples and the OD<sub>600nm</sub> and OD<sub>340nm</sub> absorbance values were monitored with time. The resulting decrease in absorbance at both wavelengths is shown in Figures 12A and 12B and it can be seen that only the pMAL31-7 sample contains a factor that reduces DCIP.--

Replace **Page 115**, last paragraph, with the following paragraph:

--The resulting sediment was suspended in SDS-PAGE sample buffer solution containing 10  $\mu$ l of 2-mercaptoethanol. Each suspension was heated at 90°C for 2 minutes, and then SDS-PAGE was performed under reducing conditions using a 12.5% gel. Following electrophoresis, the product was transferred from polyacrylamide gel to a nitrocellulose film (BIO-RAD). Western

blotting was performed using the polyclonal anti-KM31-7 antibody described in Example 1, part (a) and the anti-KM31-7 monoclonal antibody was determined to specifically precipitate KM31-7 protein from COS-1/pSR $\alpha$ 31-7 serum-free culture supernatant.--

Please replace **Pages 127 to 140** (Sequence Listing) with the replacement pages 127 to 140 of the Sequence Listing attached hereto.

**IN THE CLAIMS:**

**48. (Amended)** A polypeptide having the sequence consisting essentially of residue numbers 4 to 437 in SEQ ID NO:2.

**55. (Amended)** A polypeptide which consists essentially of amino acid numbers 1 to 526 of SEQ ID NO: 12, and which catalyzes the reduction of dichloroindophenol and oxidized glutathione.

**60. (Amended)** A polypeptide having the sequence consisting essentially of -23 to 526 of SEQ ID NO: 12.

**61. (Amended)** A method for the prophylaxis or treatment of conditions caused by, or related to, oxidative stress, or a

disease caused by activated oxygen, comprising administering to a mammal in need thereof a pharmaceutically effective, non-toxic dose of a peptide comprising the amino acid sequence consisting essentially of amino acid numbers 1 to 526 of SEQ ID NO: 12, and which catalyzes the reduction of dichloroindophenol and oxidized glutathione.

63. (Amended) A method for the prophylaxis or treatment of arteriosclerosis, diabetes, ischemic disorders, edema, vascular hyperpermeability, inflammation, gastric mucosa disorders, acute pancreatitis, Crohn's disease, ulcerative colitis, liver disorders, Paraquat's disease, pulmonary emphysema, chemocarcinogenesis, carcinogenic metastasis, adult respiratory distress syndrome, disseminated intravascular coagulation, cataracts, premature retinopathy, auto-immune diseases, porphyremia, hemolytic diseases, Mediterranean anemia, Parkinson's disease, Alzheimer's disease, epilepsy, ultraviolet radiation disorders, radioactive disorders, frostbite or burns, comprising administering to a mammal in need thereof a pharmaceutically effective, non-toxic dose of a peptide which comprises the amino acid sequence consisting essentially of amino acid numbers 1 to 526 of SEQ ID NO: 12, and which catalyzes the reduction of dichloroindophenol and oxidized glutathione.

71. (Amended) An antibody which specifically recognizes KM31-7 protein.

73. (Amended) The antibody of claim 71, wherein said antibody is humanized.

77. (Amended) A process for the purification of KM31-7 protein comprising contacting the antibody of claim 71 with a suspension containing KM31-7 protein to bind said protein.

80. (Amended) A polypeptide comprising the sequence consisting essentially of residues 4 to 437 of SEQ ID NO: 2.

Please cancel claims 1 to 47, 49 to 54, 56 to 59, 62, 64 to 70, 76 and 81 to 88, without prejudice.

Please add the following claims:

-- 89. (New) The method of claim 63, wherein the method is for the prophylaxis of arteriosclerosis, diabetes or ischemic disorders.

90. (New) The method of claim 63, wherein the method is for the treatment of arteriosclerosis, diabetes or ischemic disorders.--

R E M A R K S

This is a Divisional Application of application Serial No. 09/167,151.

In the March 7, 2000 Office Action in parent application Serial No. 09/167,151, there was a Restriction Requirement under 35 USC 121 involving Groups I to IV. Group I was elected in response to said Restriction Requirement in application Serial No. 09/167,151. The claims in this Divisional application are directed to the claims of non-elected Groups II to IV.

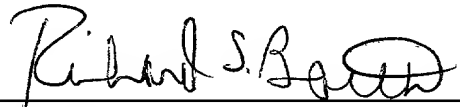
The specification was editorially revised.

A MARKED UP VERSION OF THE AMENDMENTS TO THE SPECIFICATION is enclosed.

Also enclosed is a MARKED UP VERSION OF THE AMENDED CLAIMS. New claims 89 and 90 contain features of original claim 62.

Enclosed is a copy of the DECLARATION UNDER 37 CFR 1.132 of Dr. Tohru TAKAHASHI, dated December 15, 1997, the original of which was filed in grandparent application Serial No. 08/500,635.

Respectfully submitted,



RICHARD S. BARTH  
REG. NO. 28,180

FRISHAUF, HOLTZ, GOODMAN, LANGER & CHICK, P.C.  
767 THIRD AVENUE - 25TH FLOOR  
NEW YORK, NEW YORK 10017-2023  
Tel. No. (212) 319-4900  
Fax No. (212) 319-5101  
RSB/ddf

- Enclosures:
- (1) Replacement specification pages 127 to 140 of the Sequence Listing
  - (2) MARKED UP VERSION OF THE AMENDMENTS TO THE SPECIFICATION
  - (3) MARKED UP VERSION OF THE AMENDED CLAIMS
  - (4) Copy of DECLARATION UNDER 37 CFR 1.132 of Dr. Tohru TAKAHASHI, dated December 15, 1997

**MARKED UP VERSION OF THE  
AMENDMENTS TO THE SPECIFICATION**

**Paragraph bridging pages 12 and 13:**

--The present invention will be illustrated with respect to the accompanying drawings, in which:

Figure 1 is a restriction enzyme map of cDNA of the NIa region isolated from CYVV-cDNA;

Figure 2 is a schematic drawing which shows the construction of plasmid pKNI5' containing a 5'-region of NIa;

Figure 3 is a schematic diagram which shows the construction of plasmid pKNI5IL containing a part of the IL-11 gene and a 5'-region of NIa;

Figure 4 is a schematic diagram which shows primers which were used to prepare the 5'IL DNA fragment, the CIN3 DNA fragment and in which the 3'-terminus of the NIa gene and the 5'-terminus of the IL-11 gene are fused;

Figure 5 is a schematic diagram which shows the fusion of the CIN3 DNA fragment and the [IL5'DNA] 5'IL DNA fragment by PCR;

Figure 6 is a schematic diagram which shows the construction of plasmid pKSUN9;

Figure 7 is a construction enzyme map of pUCKM31-7;

Figure 8 is a comparative diagram of the nucleotide sequences of the 3' terminals in pUCKM31-7 and pCD-31;

Figure 9 is a construction diagram of [pSR  $\alpha$ 31-7]  
pSR $\alpha$ 31-7;

Figure 10 is a schematic diagram [of] showing the introduction of a histidine hexamer encoding sequence into pUCKM31-7;

Figure 11 is a construction diagram [of] for pMAL31-7;

[Figure 12 is a diagram] Figures 12A and 12B are graphs showing the results of the assay of dichlorophenol-indophenol reducing activity; and

Figure 13 is a graph showing the determination of oxidized glutathione reducing activity.--

**Paragraph bridging pages 99 and 100:**

--The next step was to verify that the several specific 60 kDa bands identified in Example 11 are the same as the polypeptide encoded by the insert of pSR $\alpha$ 31-7. It was also desired to determine the N-terminal amino acid sequence of this polypeptide. Accordingly, a clone was prepared wherein an extra six His residues were encoded for the C-terminal of the polypeptide before the stop codon. Histidine residues have a high affinity for Ni<sup>2+</sup> and the objective was to express a polypeptide having a histidine hexamer (6 x His), which could be purified using an affinity resin column charged with [Ni<sup>2+</sup>] Ni<sup>2+</sup>.--

**Paragraph bridging pages 105 and 106:**

--90.4  $\mu$ g, as determined using the Protein Assay Kit (Bio-Rad), of each of the chromatography samples obtained in ii) above were separately mixed with 1 ml of 50  $\mu$ M DCIP (Sigma). 15  $\mu$ l of 1 mM NADPH (Boehringer-Mannheim) were then added to each of the samples and the OD<sub>600nm</sub> and OD<sub>340nm</sub> absorbance values were monitored with time. The resulting decrease in absorbance at both wavelengths [as] is shown in [Figure 12] Figures 12A and 12B, and it can be seen that only the pMAL31-7 sample contains a factor that reduces DCIP.--

Page 115, last paragraph:

--The resulting sediment was suspended in SDS-PAGE sample buffer [solution] solution containing 10  $\mu$ l of 2-mercaptoethanol. Each suspension was heated at 90°C for 2 minutes, and then SDS-PAGE was performed under reducing conditions using a 12.5% gel. Following electrophoresis, the product was transferred from polyacrylamide gel to a nitrocellulose film (BIO-RAD). Western blotting was performed using the polyclonal anti-KM31-7 antibody described in Example 1, part (a) and the anti-KM31-7 monoclonal antibody was determined to specifically precipitate KM31-7 protein from COS-1/pSR $\alpha$ 31-7 serum-free culture supernatant.--

MARKED UP VERSION OF THE AMENDED CLAIMS

48. (Amended) A polypeptide having the sequence [given by]  
consisting essentially of residue numbers 4 to 437 in [sequence]  
SEQ ID [number] NO:2 [in the sequence listing].

55. (Amended) [The] A polypeptide [encoded by the  
polynucleotide sequence of claim 49] which consists essentially  
of amino acid numbers 1 to 526 of SEQ ID NO: 12, and which  
catalyzes the reduction of dichloroindophenol and oxidized  
glutathione.

60. (Amended) [The] A polypeptide [encoded by the  
polynucleotide sequence of claim 54] having the sequence  
consisting essentially of -23 to 526 of SEQ ID NO: 12.

61. (Amended) A method for the prophylaxis or treatment of  
conditions caused by, or related to, oxidative stress, or [any] a  
disease caused by activated oxygen, comprising [the  
administration] administering to a mammal in need thereof [an] a  
pharmaceutically effective, non-toxic dose of a peptide [encoded

by the polynucleotide sequence of claim 49] comprising the amino acid sequence consisting essentially of amino acid numbers 1 to 526 of SEQ ID NO: 12, and which catalyzes the reduction of dichloroindophenol and oxidized glutathione.

63. (Amended) A method for the prophylaxis or treatment of arteriosclerosis, diabetes, ischemic disorders, edema, vascular hyperpermeability, inflammation, gastric mucosa disorders, acute pancreatitis, Crohn's disease, ulcerative colitis, liver disorders, Paraquat's disease, pulmonary emphysema, chemocarcinogenesis, carcinogenic metastasis, adult respiratory distress syndrome, disseminated intravascular coagulation, cataracts, premature retinopathy, auto-immune diseases, porphyremia, hemolytic diseases, Mediterranean anemia, Parkinson's disease, Alzheimer's disease, epilepsy, ultraviolet radiation disorders, radioactive disorders, frostbite or burns, comprising [the administration] administering to a mammal in need thereof [an] a pharmaceutically effective, non-toxic dose of a peptide [encoded by the polynucleotide sequence of claim 49] which comprises the amino acid sequence consisting essentially of amino acid numbers 1 to 526 of SEQ ID NO: 12, and which catalyzes the reduction of dichloroindophenol and oxidized glutathione.

71. (Amended) An antibody [or an equivalent thereof,] which specifically recognizes KM31-7 protein[, or which specifically recognizes a mutant or variant of KM31-7 protein].

73. (Amended) The antibody of claim 71, wherein said antibody [antigenically resembles a human antibody] is humanized.

77. (Amended) A process for the purification of KM31-7 protein comprising [the use of] contacting the antibody of claim 71 with a suspension containing KM31-7 protein to bind said protein.

80. (Amended) A polypeptide comprising the sequence [given by] consisting essentially of residues 4 to 437 [in sequence] of SEQ ID [number] NO: 2 [, or a mutant or variant thereof].

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Dorothy DeFrancesco

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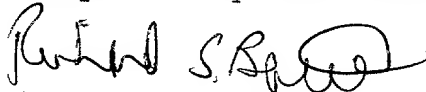
Assistant Commissioner for Patents  
Washington, D.C. 20231

S I R :

Fig. 12 was amended to separately label Figs. 12A  
and 12B. A red inked, marked up copy of Fig. 12 is enclosed.

Submitted herewith are 13 sheets of Formal Drawings  
containing Figs. 1 to 13. Please substitute the enclosed  
drawings for Figs. 1 to 13 as originally in the grandparent  
application Serial No. 08/500,635.

Respectfully submitted,



RICHARD S. BARTH  
REG. NO. 28,180

FRISHAUF, HOLTZ, GOODMAN, LANGER & CHICK, P.C.  
767 THIRD AVENUE - 25TH FLOOR  
NEW YORK, NEW YORK 10017-2023  
Tel. No. (212) 319-4900  
Fax No. (212) 319-5101  
RSB/ddf

Encs.: (1) Formal Drawings for Figs. 1 to 13 (thirteen sheets)  
(2) Red inked, marked up copy of Fig. 12



**DCIP**

600nm

Min

PMAL

PMAL31

Time (Min)	PMAL (Absorbance)	PMAL31 (Absorbance)
0	0.45	0.47
15	0.45	0.41
30	0.45	0.37

**DCIP**

Legend: PMAL (squares), PMAL31 (circles)

Time (Min)	PMAL (340nm)	PMAL31 (340nm)
0	0.205	0.197
15	0.202	0.166
30	0.200	0.135

~~FIGURE~~ 12B